PATENT

Christmann, et al.

Application No.: 09/654,293

Page 2

(iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce the reconstructed chicken zygote or oocyte;

- (v) activating the reconstructed zygote or oocyte; and
- (vi) allowing the reconstructed zygote or oocyte to develop to term.
- 2. The method of claim 1, in which the nuclear material of the recipient cell is visualized with near-infrared light using two photon laser scanning microscopy.
- 3. The method of claim 1, wherein the light has a wavelength from about 700 nm to about 1000 nm.
- 4. The method of claim 1, wherein the recipient cell is enucleated through the use of laser-mediated ablation.
- 5. The method of claim 1, wherein the visualization and enucleation are conducted using two photon laser scanning microscopy.
- 6. The method of claim 1, wherein the donor nucleus is genetically modified.

Please cancel claim 11.

Please amend claim 14 as follows:

- 14. (amended twice) A method of producing a cloned chicken comprising the steps of:
- (i) providing a recipient cell selected from the group consisting of chicken oocytes arrested at metaphase II and pronuclear zygotes;
- (ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;
 - (iii) enucleating the recipient cell using light in the near infrared region;

Br

<

PATENT

Christmann, et al.

Application No.: 09/654,293

Page 3

(iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed chicken zygote or oocyte;

- (v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;
- (vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and
 - (vii) allowing the reconstructed zygote or oocyte to develop to term.
- 15. The method of claim 14, wherein the light has a wavelength from about 700 nm to about 1000 nm.
- 16. The method of claim 14, wherein the recipient cell nucleus is visualized using two photon laser scanning microscopy.
- 17. The method of claim 14, wherein the recipient cell is enucleated using two photon laser scanning microscopy.
- 18. The method of claim 14, wherein the visualization and enucleation are conducted using two photon laser scanning microscopy.

Please amend claim 19 as follows:

- 19. (amended twice) A method of producing a transgenic chicken comprising the steps of:
- (i) providing a chicken recipient cell selected from the group consisting of chicken oocytes arrested at metaphase II and pronuclear zygotes;
- (ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;
 - (iii) enucleating the recipient cell;

COX

B3

Christmann, et al.

Application No.: 09/654,293

Page 4

- (iv) introducing a transgenic chicken donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed chicken zygote or oocyte;
 - (v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;
- (vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and
 - (vii) allowing the reconstructed zygote or oocyte to develop to term.

Please amend claim 20 as follows:

20. (amended) The method of claim 19, wherein the transgene codes for a protein selected from the group consisting of human growth hormone, interferon, β-casein, α-1 antitrypsin, antithrombin III, collagen, factor VIII, factor IX, factor X, fibrinogen,hyaluronic acid, insulin, lactoferrin, protein C, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), tissue-type plasminogen activator (tPA), feed additive enzymes, somatotropin, chymotrypsin, monoclonal antibodies, and polyclonal antibodies.

Please amend claim 21 as follows:

- 21. (amended twice) A method of producing a protein, comprising:
- (i) producing a transgenic chicken according to the method of claim 19 wherein the transgene encodes an exogenous protein, said protein being deposited into the developing eggs of said chicken;
 - (ii) harvesting hard shell eggs of said chicken; and
 - (iii) isolating the exogenous protein from said eggs.
- 22. The method of claim 21 wherein the exogenous protein is selected from the group consisting of human growth hormone, interferon, β-casein, α-1 antitrypsin, antithrombin III, collagen, factor VIII, factor IX, factor X, fibrinogen,hyaluronic acid, insulin, lactoferrin, protein C, erythropoietin (EPO), granulocyte colony-stimulating factor (G-

B3

Christmann, et al.

Application No.: 09/654,293

Page 5

CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), tissue-type plasminogen activator (tPA), feed additive enzymes, somatotropin and chymotrypsin.

Please amend claim 24 as follows:

24. (amended) A method of claim 14 wherein the cloned chicken is a knock-out or knock-in chicken.

Please amend claim 25 as follows:

25. (amended) An intact hard shell egg produced by the method of claim 21 containing exogenous protein.

Please amend claim 26 as follows:

26. (amended) A reconstructed chicken embryo comprising a nucleus from a first donor cell in the cytoplast of a second suitable recipient cell.

Please cancel claim 27.

Please amend claim 28 as follows:

- 28. (amended) A method of producing a cloned chicken comprising:
 - (i) producing a reconstructed zygote by the method of claim 1;
 - (ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and
 - (iii) allowing the reconstructed zygote to develop to term.

Please cancel claim 29.

Please amend claim 30 as follows:

- 30. (amended) A method of producing a cloned chicken comprising:
 - (i) producing a reconstructed oocyte by the method of claim 1;

PI

B5

06